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Sedative and anxiolytic effects of ethanolic extract of *Calotropis gigantea* (Asclepiadaceae) leaves

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PEER REVIEW

Peer reviewer

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Comments

This is a valuable research work in which authors have demonstrated the sedative and anxiolytic effects of crude ethanolic effects of *C. gigantea* in male Swiss albino mice. The activity was assessed based on motor activity and exploratory behavior in hole cross and open field tests. *C. gigantea* was found to have promising sedative and anxiolytic action in mice models.

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ABSTRACT

Objective: To evaluate possible anxiogenic activity, sedative property and anxiolytic potential of crude ethanolic extract of *Calotropis gigantea* leaves.

Methods: The anxiogenic activity of crude ethanolic extract of *Calotropis gigantea* leaves was evaluated using standard animal behavioral models, such as hole cross and open field; sedative property and anxiolytic potential were evaluated by conducting thiopental sodium induced sleeping time tests and elevated plus-maze test.

Results: The crude ethanolic extract exhibited a significant ($P<0.05$, $P<0.001$) decrease of motor activity and exploratory behavior in hole cross and open field tests. The extract also markedly increased both the number of visits to and time spent in the corners of the open field. The extract treated rats spent more time in the open arm of elevated plus-maze, showing its antianxiety activity. There was a decrease in the locomotor activity.

Conclusions: The obtained results provide support for the use of this species in traditional medicine and warrant further investigation to isolate the specific components that are responsible for the sedative and anxiolytic effects. Components from this plant may have a great potential value as medicinal agents, as leads or model compounds for synthetic or semi synthetic structure modifications and optimization, and as neuropharmacological probes.

KEYWORDS

CNS-depressant, Anxiolytic, *Calotropis gigantea*, Locomotor activity

1. Introduction

Currently herbal drugs are wide-spoken as green medicine for their safe and dependable health care paradigms. The traditional herbal medicines drawn an uprising attention since couple of decades due to their incredible pharmacological activities, economic viability and less side effects in different healthcare managements[1].

Drugs acting on the central nervous system (CNS) are still the most widely used pharmacological agents[2]. Commonly used CNS depressants are barbiturates, benzodiazepine and ethanol. Both barbiturates and benzodiazepines give their CNS effect by interaction with postsynaptic gamma aminobutyric acid receptor ($GABA_A$ receptor)[3]. The most serious drawback of barbiturates as a depressant is related to their narrow margin of safety, and only 10 times of their therapeutic dose may be lethal[4]. Moreover barbiturates can produce both psychological and physiological dependence[5,6]. Benzodiazepines are the most commonly used CNS depressant which lead to tolerance and physical dependence, for example diazepam typically produces sedation at dose of 5 to 10 mg in a first-time user, but

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those who repeatedly use it may become tolerant to doses of several hundred milligrams[7]. Ethanol exhibits its depressant action by changing membrane fluidity and interaction with the GABA system[4,8]; also it has a tolerance and physical dependence effect. Alcohol addiction in American society is 5% to 10% for men and 3% to 5% for women[9]. A natural CNS depressant with reduced or no toxicity is therefore, essential.

Some medical plants have been used for a wide variety of purposes such as food preservation, pharmaceutical, alternative medicine, and natural therapies for thousands of years. It is generally considered that compounds produced naturally, rather than synthetically, will be biodegraded more easily and therefore being more environmentally acceptable.

Calotropis gigantea (*C. gigantea*) R. Br. (Asclepiadaceae), is a common wild weed and locally known as Boro Akanda. It grows up to 4 m in height and possesses sessile leaves. The leaves are 10 cm in length and 8 cm in width. Its flowers are 14-15 mm long and 3-4.5 cm in diameter. The plant has oval, light green leaves, milky stem and clusters of waxy flowers that are either white or lavender in color.

Recently *C. gigantea* is scientifically reported for several medicinal properties *viz*., the flowers are reported to possess analgesic activity[10], antimicrobial and cytotoxic activity[11]. Leaves and areal parts of the plant are reported for anti-*Candida* activity[12], antibacterial activity[13] and antioxidant activity[14]. Roots are reported to contain cytotoxic activity[15], antimicrobial activity[16], insecticidal activity[17], wound healing activity[18] and CNS activity[10]. Latex of the plant is reported to contain wound healing activity[19] and antimicrobial activity[20].

It is a well-known herbal drug used to treat diversified physiological conditions. It is worthwhile validating the pharmacological properties of *C. gigantea*, which will substantiate the use of this plant over various countries for medicinal purposes. *C. gigantea* is of tremendous potentials, which deserve special attention of the scientific fraternity.

2. Materials and methods

2.1. Drugs and chemicals

Diazepam was given as a gift sample from Square Pharmaceuticals Ltd., Bangladesh. Thiopental sodium and Tween 80 were purchased from local chemical market.

2.2. Collection and extraction of C. gigantea leaves

Mature leaves of *C. gigantea* (1 000 g) were collected from a local market. The leaves were identified by Bangladesh Council of Science and Industrial Research, Chittagong, Bangladesh. After cleaning, the leaves were air dried. About 500 g of dried powder was cold extracted with ethanol for 15 d. The crude extract was then filtered, then the beaker containing extract was placed in a water bath (at 40 °C-50 °C) to evaporate the solvent from the extract, resulting in a semi-solid extract.

2.3. Animal

Male Swiss albino mice, 3-4 weeks old, weighing between 20-25 g, were collected from the the International Center for Diarrheal Disease and Research, Bangladesh. Animals were maintained under standard environmental conditions [temperature: (24±1) °C, relative humidity: 55%-65% and 12 h light/12 h dark cycle] and had free access to feed and water *ad libitum*. Prior to experimentation, the animals were acclimatized to laboratory condition for one week. Authoritative approval for the experiment was given by the Ethics Committee of the institution.

2.4. Neuropharmacological activities

The neuropharmacological activities of crude ethanolic extract of *C. gigantea* leaves were estimated by hole cross test, open field test, elevated plus-maze (EPM) test and thiopental sodium induced sleeping time test. During each experiment, male Swiss albino mice were divided into three groups, namely, control, positive control and test sample. Each group containing 5 mice were treated as the following arrangement: control, 1% v/v Tween-80 in water, 0.5 mL/mice; positive control, diazepam, 1 mg/kg body weight; test sample, ethanol extract at the dose of 400 mg/kg body weight.

2.4.1. Hole cross test

To conduct this test, a steel partition (30×20×14 cm) was fixed in the middle of a cage. A hole with 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90, and 120 min after oral administration of the test drugs[21].

2.4.2. Open field test

The experiment was carried out to determine depressive action of the test drugs on CNS in mice. The floor of an open field of half square meter was divided into a series of squares each alternatively colored with black and white. The apparatus was 40 cm high. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90, and 120 min after oral administration of the test

drugs[22].

2.5. EPM test

The anxiolytic activity of plant extracts was evaluated using the EPM test. The apparatus was situated 40 cm above the floor, consisting of two open arms (5×10 cm) and two closed arms (5×10×15 cm) radiating from a platform (5×5 cm) to form a plus-sign figure. The open arms edges were 50 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. Sixty minutes after administration of the test drug, each animal was placed at the center of the maze facing one of the enclosed arms. During the 5 min test period, the number of open and enclosed arms entries, plus the time spent in open and enclosed arms, was recorded via the method of Pillow and File[23]. Entry into an arm was defined as the point when the animal places all four paws onto the arm. The procedure was conducted in a sound attenuated room; observations were made from an adjacent corner.

2.6. Thiopental sodium induced sleeping time test

The animals were randomly divided into ten groups consisting of five mice each. The test groups received ethanolic extract of *C. gigantea* leaves at the dose of 400 mg/kg body weight while positive control was treated with diazepam (1 mg/kg) and control with vehicle (1% Tween 80 in water). Thirty minutes later, thiopental sodium (40 mg/kg) was administered to each mouse to induce sleep. The onset time of sleep was noted for all the animals. After induction of sleep, mice were placed in the inverted position and when sedation was over, the mice came to normal posture and time was noted. According to the method of Ferrini *et al.*, the interval between loss and recovery of righting reflex was used as index of hypnotic effect and the time interval between injection of thiopentone sodium and start of sleep was recorded as latency time[24].

2.7. Statistical analysis

Statistical analysis for animal experiment was carried out using One-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group, in which $P<0.05$, 0.001 were considered to be statistically significant.

3. Results

Ethanolic extract of *C. gigantea* showed significant ($P<0.05$, $P<0.001$) decrease of movement from its initial value at 0 to 120 min which was comparable with that of the group treated with diazepam (Figure 1).

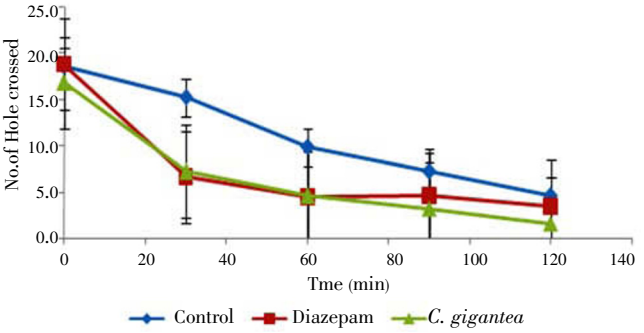

Figure 1. Effect of the ethanol extract of *C. gigantea* leaves on hole cross test in mice.

In the open field test, the number of squares traveled by the mice was suppressed significant ($P<0.05$, $P<0.001$) from its initial value at 0 to 120 min (Figure 2).

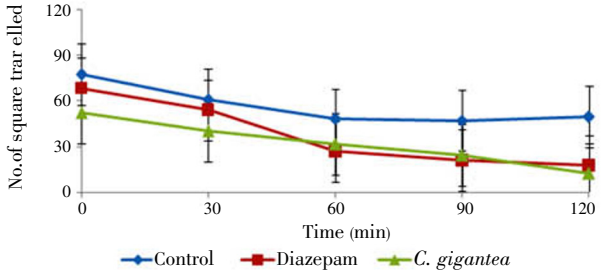

Figure 2. Effect of the ethanol extracts of *C. gigantea* leaves on open field test in mice.

Thiopental sodium induced sleeping time test showed that ethanolic extract of *C. gigantea* leaves induced sleep at an earlier stage, *i.e.* it had a good effect on the onset of thiopental sodium induced sleep and also lengthened the duration of sleeping time in test animals compared to control (Table 1).

Table 1

Effects of the ethanolic extract of *C. gigantea* leaves on thiopental induced sleeping time in mice. Values are mean±SEM (n=5).

Treatment	Dose (mg/kg)	Onset of sleep (min)	Duration of sleep (min)
Control	Vehicle	40.200±1.655	47.000±0.949
Diazepam	1	14.800±0.860**	149.800±3.441**
*C. gigantea*n	400	4.200±0.860**	152.200±6.344**

* $P<0.05$, ** $P<0.001$, Dunnet's test as compared to control (vehicle: 0.4 mL/mouse, *p.o.*).

EPM test showed significant ($P<0.05$-0.001) reduction in the percentage of entries of mice into the open arms and the percentage of time spent in the open arms of the EPM (Table 2).

Table 2

Effects of the ethanolic extract of *C. gigantea* leaves on the percentage of entries and the time spent in open arms of the elevated plus-maze during the 5-min test session [Values are mean ± SEM, (n = 5)]

Treatment	Dose (mg/kg)	% Entry into open arm	% Time spent in open arm
Control	Vehicle	55.88±1.908	51.93±7.372
Diazepam	1	76.28±1.652*	79.39±5.182*
*Calotropis gigantea*n	400	67.95±1.565*	75.81±5.682

* $P<0.05$, ** $P<0.001$, Dunnet's test as compared to control (vehicle: 0.4 mL/mouse, *p.o.*).

4. Discussion

Despite intensive efforts to develop novel psychiatric drugs for anxiety and depression disorders over the past two decades, all drugs have so far failed to minimize side effects. In this respect, herbal medicines could be an attractive candidate as the therapeutic strategies for these conditions[25]. A major role for plant-derived compounds based on the reported immunomodulatory effects has emerged in recent times and has led to the rigorous scientific examination to determine efficacy and safety[26].

The result of hole cross and open field tests showed that the studied plant decreased the frequency as well as the bountifulness of movements. Since the level of excitability of the CNS is measured by locomotor activity, this reduction in spontaneous motor activity that could be considered as the sedative effect of the plant extracts. The locomotor activity lowering effect was evident at the 2nd observation (30 min) and continued up to the 5th observation period (120 min).

The above result showed that crude ethanolic extracts of *C. gigantea* plant had strong sedative and hypnotic activity that principally mediated in the CNS by the $GABA_A$ receptor complex. Thiopental, a barbiturate drug, produce sedative-hypnotic effect at a certain dose due to their interaction with $GABA_A$ receptors which enhances the GABAergic transmission. It potentiates GABA activity, entering chloride into the neuron by prolonging the duration of chloride channel opening. On the other hand, thiopental can block excitatory glutamate receptors. All of these molecular action lead to decrease of neuronal activity that support the following reference substances which possess sedative action.

However, the anxiolytic activity of the ethanolic extract of *C. gigantea* was measured by using EPM suggested when the test drug increases open arms entries without altering the total number of arm entries. Diazepam has been used as a standard anxiolytic and also frequently employed in behavioral pharmacology as a reference compound of potentially anxiolytic-acting substances. But the fractions of plant extract at 400 mg/kg body weight in mice showed significant increase in the percentage of entries into open arms and time spent in the open arms of the maze.

Analyzing the results of present study, it can be inferred that the crude ethanolic extract of *C. gigantea* possess strong sedative and anxiolytic activity. Therefore, this extract could be considered for the treatment of anxiety and related neuropsychiatric disorders by conducting further pharmacological studies and mechanism of sedative and anxiolytic action, as well as to identify the active compound(s) responsible for this bioactivity in the animal model.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Anxiety and depressive disorders are the most frequent psychiatric conditions encountered today. It is reported that more than 20% of the adult population suffer from these conditions at some stage during their life. A natural CNS depressant with reduced or no toxicity is therefore, essential.

Research frontiers

The present work depicts the evaluation of possible anxiogenic activity, sedative property and anxiolytic potential of crude ethanolic extract of *C. gigantea* leaves. The obtained results provide support for the use of this species in traditional medicine and warrants further pharmacological investigations that could lead to novel leads.

Related reports

The roots of the plant have been reported to contain CNS activity by Pathak and Argal (2006). Furthermore, folklore medicine has evidence of effectiveness of herbs in treating various disorders.

Innovations and breakthroughs

Results of the present study inferred that the crude

ethanolic extract of the leaves of *C. gigantea* possess strong sedative and anxiolytic activity.

Applications

This extract could be considered for the treatment of anxiety and related neuropsychiatric disorders after conducting further pharmacological studies and elucidating the mechanism of sedative and anxiolytic action, as well as to identify the active compound(s) responsible for this bioactivity in the animal model.

Peer review

This is a valuable research work in which authors have demonstrated the sedative and anxiolytic effects of crude ethanolic effects of *C. gigantea* in male Swiss albino mice. The activity was assessed based on motor activity and exploratory behavior in hole cross and open field tests. *C. gigantea* was found to have promising sedative and anxiolytic action in mice models.

References

[1] Chew AL, Jessica JJ, Sasidharan S. Antioxidant and antibacterial activity of different parts of *Leucas aspera*. *Asian Pac J Trop Biomed* 2012; **2**(3): 176-180.

[2] Katzung BG. *Basic and clinical pharmacology*. 6th ed. California: Prentice-Hall International In.; 1994, p. 323.

[3] Rang HP, Dale MM, Ritter JM. *Pharmacology*. 3rd ed. London: Churchill Livingstone Inc.; 1996, p. 512.

[4] Clark WG, Brater DC, Johnscn AR. *Goth's medical pharmacology*. 12th ed. New Delhi: Galgotia Publication Pvt. Ltd.; 1989, p. 288.

[5] Essig CF. Addiction to nonbarbiturate sedative and tranquilizing drug. *Clin Pharmacol Ther* 1964; **5**: 334-343.

[6] Isbell H, Fraser HF. Addiction to analgesics and barbiturates. *J Pharmacol Exp Ther* 1950; **99**(4:2): 355-397.

[7] O'Brien CP. Drug addiction and drug abuse. In: Brunton L, Chabner B, Knollman B, editors. *Goodman and Gilman's the pharmacological basis of therapeutics*. 9th ed. New York: McGraw-Hill Professional; 1996, p. 570.

[8] Tripathi KD. *Essential medical pharmacology*. 3rd ed. New Delhi: Jaypee Brothers Medical Publishers; 1994, p. 324.

[9] Schuckit MA. A low level of response to alcohol as a predictor of future alcoholism. *Am J Psychiatry* 1994; **151**: 184-189.

[10] Argal A, Pathak AK. CNS activity of *Calotropis gigantea* roots. *J Ethnopharmacol* 2006; **106**(1): 142-145.

[11] Habib MR, Karim MR, Antimicrobial and cytotoxic activity of di-(2-ethylhexyl) phthalate and anhydrosophoradiol-3-acetate isolated from *Calotropis gigantea* (Linn.) flower. *Mycobiology* 2009; **37**(1): 31-36.

[12] Kumar G, Karthik L, Bhaskara Rao KV. *In vitro* anti-*Candida* activity of *Calotropis gigantea*. *J Pharm Res* 2010; **3**(3): 539-542.

[13] Kumar G, Karthik L, Bhaskara Rao KV. Antibacterial activity of aqueous extract of *Calotropis gigantea* leaves-an *in vitro* study. *Int J Pharm Sci Rev Res* 2010; **4**(2): 141-144.

[14] Singh N, Jain NK, Kannojia P, Garud N, Pathak AK, Mehta SC. *In vitro* antioxidant activity of *Calotropis gigantea* hydroalcohlic leaves extract. *Der Pharmacia Lettre* 2010; **2**(3): 95-100.

[15] Wang Z, Wang M, Mei W, Han Z, Dai H. A new cytotoxic pregnanone from *Calotropis gigantea*. *Molecules* 2008; **13**(12): 3033-3039.

[16] Alam MA, Habib MR, Nikkon R, Rahman M, Karim MR. Antimicrobial activity of akanda (*Calotropis gigantea* L.) on some pathogenic bacteria. *Bangladesh J Sci Ind Res* 2008; **43**(3): 397-404.

[17] Alam MA, Habib MR, Nikkon F, Khalequzzaman M, Karim MR. Insecticidal activity ofroot bark of *Calotropis gigantea* L. against *Tribolium castaneum* (Herbst). *World J Zool* 2009; **4**(2): 90-95.

[18] Deshmukh PT, Fernandes J, Aarte A, Toppo E. Wound healing activity of *Calotropis gigantea* root bark in rats. *J Ethnopharmacol* 2009; **125**(1): 178-181.

[19] Nalwaya N, Pokharna G, Deb L, Jain NK. Wound healing activity of latex of *Calotropis gigantea*. *Int J Pharm Pharm Sci* 2009; **1**(1): 176-181.

[20] Kumar G, Karthik L, Bhaskara Rao KV. Antimicrobial activity of latex of *Calotropis gigantea* against pathogenic microorganisms-an *in vitro* study. *Pharmacologyonline* 2010; **3**(3): 155-163.

[21] Subhan N, Alam MA, Ahmed F, Shahid IJ, Nahar L, Sarker SD. Bioactivity of *Excoecaria agallocha*. *Braz J Pharm* 2008; **18**: 521-526.

[22] Hawiset T, Muchimapura S, Wattanathorn J, Sripanidkulchai B. Screening neuropharmacological activities of *Kaempferia parviflora* (Krachai dam) in healthy adult male rats. *Am J Appl Sci* 2011; **8**: 695-702.

[23] Pellow S, File S. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. *Pharmacol Biochem Behav* 1986; **24**: 525-529.

[24] Ferrini R, Miragoli G, Taccardi B. Neuro-pharmacological studies on SB 5833, a new psychotherapeutic agent of the benzodiazepine class. *Arzneimittelforschung* 1974; **24**: 2029-2032.

[25] Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Braz J Med Biol Res* 2000; **33**: 179-189.

[26] Licciardi PV, Underwood JR. Plant-derived medicines: a novel class of immunological adjuvants. *Int Immunopharmacol* 2011; **11**(3): 390-398.